Nanostructured Electrodes with the Enhanced Output Signal for Biosensor Applications

Maly J., Krejčí J., Jakubka L., Pilloton R., Sameh K., Steffan P., Sugiura M.
Overview of the presented work

- The immobilization method of bioactive molecules on the surface of transducer plays an important role in functionality of biosensors, namely in such functional parameters as long term stability, sensitivity, reproducibility and the magnitude of output signal.

- The immobilized structure itself can significantly decrease the mass transfer between active part of biorecognition element and electrode of electrochemical sensor.

- Here we present a case study based on the behaviour of the oriented monolayer of histidine tagged photosystem II on gold electrode which shows that the electrode with nanostructured surface could significantly improve the mass transfer in the immobilized bioactive layer with the consequence of a higher output signal of sensor.
Main goals of the presented work

We aimed to show that:

• The densely packed bioactive molecules can act as the barrier to the mediator diffusion towards the electrode;

• The functionality can be enhanced by spacer in the bioactive layer;

• The enzyme or generally the bioactive layer could work more efficiently on nanostructured electrodes;

• There is possibility to find the time window where mass transport towards the nanostructured electrode is higher than towards the planar electrode.
We have recently designed a biosensor for detection of herbicides based on isolated PSII from thermophilic cyanobacteria *Synechococcus elongatus* (Koblizek et al. 2002)

**We have used such biosensor for electron transport study using:**

- Cross-linked photosystem II (PSII) in bovine serum albumine (BSA) – glutaraldehyde (GA) gel matrix.

- Histidine tagged PSII (His-tag PSII) immobilized in the form of monolayer on the predeposited self-assembled monolayer of nitrilotriacetic acid chelator (NTA) on gold electrode (Maly et al. 2002).
Synthesis of self assembled monolayer of Ni-NTA chelator on gold electrode

(i) selfassembled monolayer (SAM) of cysteamin is deposited on gold electrode, modified (ii) with glutaraldehyde and finally with nitrilotriacetic acid (NTA) (iii) (Maly J. et al. 2002).
Electrochemical system using oxygen evolving PSII particles from the cyanobacterium *Synechococcus elongatus*

**Principle**

Electron transport from PSII with functional OEC complex to the platinum working electrode mediated using the artificial electron acceptor duroquinone (DQ). Reduced duroquinone is reoxidised at +620mV (vs. Ag/AgCl reference electrode). Oxidation current is proportional to the electron transport activity of PSII. Electric current generated by PSII is lower in the presence of herbicide. Screen-printed Pt:Ag/AgCl electrodes in three electrode arrangements are used.
The mass transport is influenced by immobilisation strategy of enzyme

Panel A:
The inner structure of BSA-GA gel matrix with immobilized photosystem II.

Panel B:
The structure of the oriented monolayer of photosystem II.
Assembling of PSII on gold electrode monitored on-line during process

Electric current response of the electrode monitored on-line during the loading of electrode with His-tagged PSII
Influence of spacer BSA biomolecule in monolayer of PSII on biosensor activity

Panel A:
The structure of oriented monolayer of PSII mixed with the BSA spacer.

Panel B:
The structure of electrode with the nano-structured active surface.
Influence of spacer BSA biomolecule in monolayer of PSII on biosensor activity

Signal obtained during the time-course of measurement from electrode with the monolayer of His-tagged PSII mixed with BSA (Panel A) and with the monolayer of pure His-tagged PSII (Panel B).
Nanostructured architecture of electrodes for improvement of mediator diffusion

The proposed parameters of the electrode with nanostructured active surface.
Comparison of time dependent current densities obtained by simulation for spherical and planar electrode

1: $R = 50 \text{ nm}$
2: $R = 100 \text{ nm}$
3: $R = 500 \text{ nm}$
4: $R = 1 \mu\text{m}$
5: $R = 5 \mu\text{m}$
6: planar electrode

$R$: radius of the nanoelectrode

\[ i = nF\left(\frac{D_0 C_0}{R_0}\right) \times \left[1 + \frac{R_0}{\sqrt{D\pi t}}\right] \]
Comparison of the current response of planar (2) and nanostructured (1) electrode

The surface of macroelectrode is $1 \text{ mm}^2$. It consists of electrodes with diameter $1 \mu\text{m}$ and distance $2 \mu\text{m}$. The time window optimal for increasing the measured output signal is marked.
Conclusions

- The nanostructure of electrodes significantly enhances the output signal of electrochemical biosensors.

- The improvement of mass transfer to the electrode has been analyzed for electrode consisting of an array electrodes of diameter in range from 50 nm to 5 µm by digital simulations.

- The improvement of mass transport through the bioactive layer was verified by co-immobilization of spacer in the self-assembled monolayer of histidine tagged photosystem II from thermophilic cyanobacteria *Synechococcus elongatus*

- The combination of both principles together with optimal time window measurement enables the enhancement of output signal by factor up to 50 in comparison with classical electrodes.

- The measurement needs the methods which suppress the double layer capacity. The pulse methods are preferred.
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